UNIVERSITI PUTRA MALAYSIA

IN VITRO PROPAGATION AND DETERMINATION OF DOSE FOR MUTATION INDUCTION IN TORCH GINGER (ETLINGERA ELATIOR JACK.)

ASNITA BINTI ABU HARIRAH

FP 2002 33

IN VITRO PROPAGATION AND DETERMINATION OF DOSE FOR MUTATION INDUCTION IN TORCH GINGER (*ETLINGERA ELATIOR* JACK.)

By

ASNITA BINTI ABU HARIRAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of Requirement for the Degree of Master of Agricultural Science

October 2002

DEDICATION

My beloved husband;

Ahmad Termizi b. Mohd. Yusof

My lovely daughters;

Aida Fatini bt. Ahmad Termizi

Alya Fakhira bt. Ahmad Termizi

My loving parents;

Hj. Abu Harirah b. Mohd. Nasif

Hjh. Teh Kalsom bt. Abd. Halid

My brothers and sister;

Aszurina bt. Abu Harirah

Asnizam b. Abu Harirah

Mohd. Asrul b. Abu Harirah

Zaili b. Hj. Hamzah

Thank you for all the valuable support, sacrifices and love

May Allah bless all of you

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Agricultural Science

IN VITRO PROPAGATION AND DETERMINATION OF DOSE FOR MUTATION INDUCTION IN TORCH GINGER (_ETLINGERA ELATIOR_ JACK.)

By

ASNITA BINTI ABU HARIRAH

October 2002

Chairman: Associate Professor Saleh bin Kadzimin, Ph.D.

Faculty: Agriculture

Mutation induction has provided an avenue for creating variability in many plant species. Its application has brought new dimension to many horticultural crops including ornamentals where creation of new varieties through conventional breeding and selection has always been difficult, costly and time consuming.

The propagation of *Etlingera elatior* or torch ginger has largely been through the use of suckers with its slow rate of multiplication. Thus, the present study is conducted to develop a protocol for rapid propagation and creation of new and better varieties by combining the techniques of *in vitro* culture and radiation mutagenesis.

In developing the protocol for rapid propagation, *in vitro* cultures of torch ginger were established by placing shoot tip explants on half and full strength MS medium containing various levels of BAP and NAA each at 1.0, 2.0, 3.0 and 4.0 mg/l and in combinations of both (BAP and NAA). The highest number of shoot multiplication was obtained from treatment with full strength MS medium supplemented with 1.0 mg/l BAP. Cultures in half strength MS medium were not significantly different amongst all treatments. Medium containing BAP alone gave superior results than those with combination of both growth regulators. Generally, the presence of NAA reduced the number of shoots.

MS medium supplemented with NAA alone was significantly different in root development except for treatments with full strength MS medium supplemented with 4.0 mg/l NAA. The highest number of roots was obtained from treatment in half strength MS medium supplemented with 1.0 mg/l NAA.

Irradiation of seeds was done using gamma rays from ^{60}Co source at levels of 10, 20, 30, 40 and 50 Gy at a dose rate of 0.225 Gy/sec. From radiosensitivity test results, a 100% survival rate was recorded from the control and 10 Gy treatments. Treatment at 20 Gy gave survival rate of 60%. There was no survival from treatments with 30, 40 and 50 Gy.

The study concluded that the optimum dose for torch ginger was between 14-22 Gy. Irradiation at levels higher than 22 Gy was highly lethal.

Except for the control, irradiation of seeds caused stunting of shoots. Although germination occurred at 30 Gy and 40 Gy treatments, shoots turned brown and later died. Increasing the irradiation dose caused a general decrease in mean value of the first leaf height. The mean value of the first leaf height of non-irradiated sample (control) was 4.58 ± 0.13 cm, while treatments at 10 Gy and 20 Gy were 2.24 ± 0.09 cm and 1.57 ± 0.19 cm, respectively.

The RAPD (Random amplified polymorphic DNA) technique was used to detect the variation of genomic DNA of mutated samples from the different irradiation doses. Among 10 different random primers from the Operon Kit A and B, only 1 primer (OPA-04) showed amplification on 9 DNA samples obtained from cultures treated with different doses of gamma irradiation. From the study, polymorphism was detected using primer OPA-04. RAPD profiles showed 1 missing band of 630 bp and 2 missing bands of 410 bp for different samples that had been treated at 20 Gy.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian

PEMBIAKAN *IN VITRO* DAN PENENTUAN DOS BAGI ARUHAN MUTASI KE ATAS TORCH GINGER (*ETLINGERA ELATIOR* JACK.)

Oleh

ASNITA BINTI ABU HARIRAH

Oktober 2002

Pengerusi: Profesor Madya Saleh bin Kadzimin, Ph.D.

Fakulti: Pertanian

Aruhan mutasi telah membuka suatu ruang baru bagi menghasilkan pelbagai variasi di dalam kebanyakan spesies tumbuhan. Hasil daripada aplikasi bidang ini telah memberikan suatu dimensi baru kepada kebanyakan tanaman hortikultur termasuklah tanaman hiasan di mana penghasilan varieti baru serta pemilihan tanaman menerusi pembiakan konvensional, lazimnya adalah sukar serta melibatkan perbelanjaan yang tinggi dan tempoh yang panjang.

Kebanyakan pembiakan *Etlingera elatior* atau 'torch ginger' menerusi penggunaan tunas sulur memberikan kadar pembiakan yang rendah. Justeru itu, kajian ini dijalankan untuk membentuk satu protokol bagi

menghasilkan pembiakan yang lebih cepat serta penghasilan varieti baru yang lebih baik dengan menggabungkan teknik kultur *in vitro* dan mutagenesis radiasi.

Di dalam membentuk protokol bagi menghasilkan pembiakan yang cepat, kultur *in vitro* torch ginger dikembangbiak dengan mengkultur eksplan mercu pucuk di dalam media separuh dan sepenuhnya nutrien MS yang mengandungi pelbagai paras BAP dan NAA yang setiap satunya pada 1.0, 2.0, 3.0 dan 4.0 mg/l dan juga kombinasi bagi kedua-dua paras hormon (BAP dan NAA). Bilangan pembiakan pucuk yang tertinggi telah dicapai bagi rawatan yang terdiri daripada media sepenuhnya nutrien MS yang ditambah dengan 1.0 mg/l BAP. Kultur yang terdiri daripada media separuh nutrien MS didapati tidak mempunyai perbezaan yang bererti terhadap pembiakan pucuk. Media yang ditambahkan dengan BAP sahaja memberikan keputusan bilangan pucuk yang tertinggi berbanding dengan medium mengandungi kombinasi kedua-dua pengawalatur tumbesaran. Umumnya, kehadiran media yang ditambah dengan NAA mengakibatkan pengurangan kepada bilangan pucuk.

Media MS yang ditambah dengan NAA sahaja memberikan bilangan akar yang tertinggi berbanding dengan rawatan bagi kombinasi kedua-dua NAA dan BAP kecuali bagi rawatan terhadap kultur yang terdiri daripada

media sepenuhnya nutrien MS yang ditambah dengan 4.0 mg/l NAA. Bilangan akar yang tertinggi telah dicapai bagi rawatan yang terdiri daripada media separuh nutrien MS yang ditambah dengan 1.0 mg/l NAA.

Radiasi ke atas biji benih telah dilakukan dengan menggunakan sinar gamma daripada sumber ^{60}Co pada paras 10, 20, 30, 40 dan 50 Gy pada kadar dos 0.225 Gy/s. Hasil daripada ujian radiosensitiviti yang telah dijalankan, didapati kadar anak pokok yang hidup telah direkodkan 100% bagi kultur kawalan dan juga rawatan biji benih yang didedahkan pada dos 10 Gy. Rawatan biji benih yang didedahkan pada dos 20 Gy telah memberikan 60% kadar hidup. Manakala pada rawatan dos 30, 40 dan 50 Gy, tiada anak pokok yang berjaya hidup.

Di samping itu juga, radiasi pada biji benih menyebabkan anak pokok terbantut tumbesarannya melainkan pada kultur kawalan. Walaupun pada rawatan radiasi dos 30 Gy dan 40 Gy menghasilkan percambahan tetapi warna pucuk bertukar menjadi perang dan akhirnya mati. Secara umumnya, setiap pertambahan radiasi dos menyebabkan pengurangan kepada min tinggi anak pokok. Min tinggi anak pokok pada sampel yang tidak didedahkan pada sumber radiasi (kawalan) adalah 4.58 ± 0.13 cm. Manakala pada sampel rawatan biji benih yang didedahkan radiasi dos

pada 10 Gy dan 20 Gy, masing-masingnya adalah 2.24 ± 0.09 cm dan 1.57 ± 0.19 cm.

Hasil daripada kajian ini dapat disimpulkan bahawa dos optima bagi torch ginger adalah di antara 14-22 Gy. Radiasi dos pada paras yang lebih tinggi daripada 22 Gy menyebabkan kematian anak pokok.

Teknik RAPD (random amplified polymorphic DNA) telah digunakan untuk mengesan variasi pada DNA genom sampel yang mutasi hasil daripada pendedahan kepada radiasi dos yang berbeza. Hasil daripada penggunaan 10 primer rambang Operon Kit A dan B, hanya 1 primer yang menunjukkan amplifikasi DNA terhadap 9 sampel DNA yang didedahkan kepada radiasi gamma iaitu primer OPA-04. Daripada kajian ini, polimorfik DNA telah dikesan oleh primer OPA-04, merujuk kepada kehilangan satu jalur DNA pada 630 bp dan juga kehilangan dua jalur DNA iaitu 410 bp pada dua sampel berlainan bagi rawatan radiasi dos 20 Gy.

ACKNOWLEDGEMENTS

My appreciation and sincere thanks to Associate Professor Dr. Saleh Kadzimin, Faculty of Agriculture, Universiti Putra Malaysia (UPM) for his guidance and supervision.

I would also like to express my gratitude to the members of my Supervisory Committee, Dr. Faridah Qamaruz Zaman, Institute of Bioscience, Universiti Putra Malaysia and Dr. Mohd. Nazir Basiran, Malaysian Institute for Nuclear Technology Research (MINT) for their encouragement and guidance in carrying out this research.

My deepest appreciation goes to Mr. Mushlim Musa a local hobbyist, Mr. Abdul Rahman Sidam, Mr. Elixon Sunian and staff of the Crop Science Department, UPM for their kind support during the study.

I also would like to thank Miss Affrida Abu Hassan, Mr. Shuhaimi Shamsudin and staff of the Plant Biotechnology Laboratory, MINT for their kindness and help throughout the study period.

Finally, my thanks are due to all my friends for sharing the moments and giving support during the study.

I certify that an Examination Committee met on 23rd October, 2002 to conduct the final examination of Asnita bt. Abu Harirah on her Master of Agricultural Science thesis entitled "*In Vitro* Propagation and Determination of Dose for Mutation Induction in Torch Ginger (*Etlingera elatior* Jack.)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

MAHERAN BINTI ABD. AZIZ, Ph.D.
Department of Crop Science
Faculty of Agriculture
Universiti Putra Malaysia
(Chairperson)

SALEH BIN KADZIMIN, Ph.D.
Associate Professor
Department of Crop Science
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

FARIDAH BINTI QAMARUZ ZAMAN, Ph.D.
Institute of Bioscience
Universiti Putra Malaysia
(Member)

MOHD NAZIR BIN BASIRAN, Ph.D.
Agrotechnology and Biosciences Division
Malaysian Institute for Nuclear Technology Research
(Member)

SHAMSHER MOHAMAD RAMADILI, Ph.D.
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 DEC 2002

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Master of Agricultural Science. The members of the Supervisory Committee are as follows:

SALEH BIN KADZIMIN, Ph.D.
Associate Professor
Department of Crop Science
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

FARIDAH BINTI QAMARUZ ZAMAN, Ph.D.
Institute of Bioscience
Universiti Putra Malaysia
(Member)

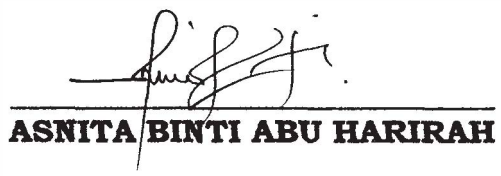
MOHD NAZIR BIN BASIRAN, Ph.D.
Agrotechnology and Biosciences Division
Malaysian Institute for Nuclear Technology Research
(Member)

AINI IDERIS, Ph.D.
Professor/Dean
School of Graduate Studies,
Universiti Putra Malaysia

Date: 13 FEB 2003

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



ASNITA BINTI ABU HARIRAH

Date: 27 DEC. 2002 .

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

2-AP	:	2-amino-purine
AFLP	:	amplified fragment length polymorphism
AP-PCR	:	arbitrary-primed PCR
ASAP	:	allele-specific associated primers
BA	:	6-benzyladenine
BAP	:	6-benzylaminopurine
bp	:	base pair
5-BU	:	5-bromo-uracil
°C	:	degree(s) Celsius
cm	:	centimetre(s)
^{60}Co	:	cobalt-60
CO_2	:	carbon dioxide
CPPU	:	N-(2-chloro-4-pyridy)-N'-phenylurea
CTAB	:	cetyl trimethylammonium bromide
2,4-D	:	2,4-dichlorophenoxyacetic acid
DAF	:	DNA amplification fingerprinting
DES	:	diethyl sulphate
DF	:	degree of freedom
DNA	:	deoxyribonucleic acid
EDTA	:	ethylenediaminetetraacetic acid
EMS	:	ethyl methanesulphonate

°F	:	degree(s) Fahrenheit
g	:	gram(s)
Gy	:	Gray(s)
Gy/sec	:	Gray(s) per second
H	:	hydrogen bond
HCl	:	hydrochloric acid
HNO_2	:	nitrous acid
IAA	:	indole-3-acetic acid
IAEA	:	International Atomic Energy Agency
IBA	:	indole-3-butyric acid
iP	:	isopentenyladenine
iPA	:	isopentenyladenosine
J	:	Joule
KCl	:	potassium chloride
kb	:	kilo base
LD	:	lethal dose
M	:	molar(s)
$MgCl_2$	:	magnesium chloride
µg	:	microgram(s)
mg/l	:	milligram(s) per litre
min	:	minute(s)
MINT	:	Malaysian Institute for Nuclear Technology Research
µl	:	microlitre(s)

ml	:	millilitre(s)
μM	:	micromolar(s)
mM	:	millimolar(s)
mm	:	millimetre(s)
mM	:	millimolar(s)
MNH	:	N-nitroso-N-methyl urea
MNU	:	N-nitroso urea
MRC	:	Molecular Research Center
MS	:	Murashige and Skoog's
NAA	:	1-naphthaleneacetic acid
NaCl	:	sodium chloride
NaOH	:	sodium hydroxide
NG	:	N-methyl-N-nitro-N-nitrosoguanidine
NH_2OH	:	hydroxylamine
nm	:	nanometre(s)
OD	:	optical density
PAA	:	phenylacetic acid
PCR	:	polymerase chain reaction
pH	:	hydrogen ion concentration
RAPD	:	random amplified polymorphic DNA
RCBD	:	Random Complete Block Design
RNA	:	ribonucleic acid
rpm	:	revolution(s) per minute

SCAR	:	sequenced characterised amplified regions
SE	:	standard error
SPAR	:	single primer amplification reaction
2,4,5-T	:	2,4,5-trichlorophenoxyacetic acid
TBE	:	Tris base-EDTA
TDZ	:	thidiazuron
TE	:	Tris-EDTA
Tris-HCl	:	Tris(hydroxymethyl) aminomethane hydrochloride
UV	:	ultraviolet
V	:	volt(s)
Z	:	zeatin
ZR	:	zeatinriboside